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PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Lockhart, Helen C.
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210
ETATS-UNIS D'AMERIQUE

Subject to PTA? YES/NO
per docket/ECB
8-8-02

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NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

	Date of mailing *(day/month/year)* 05.08.2002
Applicant's or agent's file reference M0656/7063WO	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US01/07464	08/03/2001	08/03/2000

Applicant
MASSACHUSETTS INSTITUTE OF TECHNOLOGY

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

For the purpose of deciding whether the claimed invention is patentable or not, the elected Offices may apply criteria additional to or different from the criteria on which the international preliminary examination report is based (see Articles 27(5), 33(5)). Additional criteria may include e.g. exemptions from patentability and the requirements of enabling disclosure and of clarity and support of claims.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Faux, K Tel.+49 89 2399-8062

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
M0656/7063WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US01/07464	08/03/2001	08/03/2000
International Patent Classification (IPC) or national classification and IPC C12N15/60		
Applicant MASSACHUSETTS INSTITUTE OF TECHNOLOGY		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 14 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
26/09/2001	05.08.2002
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Mundel, C Telephone No. +49 89 2399 7314

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US01/07464

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-69	as originally filed				

Claims, No.:

1-19,28-60	as originally filed				
20-27	as received on	15/07/2002	with letter of	11/07/2002	

Drawings, sheets:

1/17-17/17	as originally filed				

Sequence listing part of the description, pages:

1-3, filed with the letter of 11.04.01

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☒ contained in the international application in written form.
   - ☒ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 28-60.

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 28-60.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.
   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US01/07464

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:
   see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-27.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	6-8, 10, 15, 18-22
	No:	Claims	1-5, 9, 11-14, 16-17 and 23-27
Inventive step (IS)	Yes:	Claims	18-19 and 21-22
	No:	Claims	1-17, 20 and 23-27
Industrial applicability (IA)	Yes:	Claims	7, 17-19 and 23-27
	No:	Claims	4-6, 9, 11, 15 and 20-22 (completely) and 1-3, 8, 10, 12-14 and 16 (partially) (see Citations and explanations)

2. Citations and explanations
   see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

A unity objection was raised by the International Search Authority (ISA). Since the applicant didn't pay additional search fees, only invention 1 (claims 1-27) has been searched and, therefore, examined.

Re Item IV

Lack of unity of invention

According to **Rule 13 PCT** an application must relate only to one invention or to a group of inventions so linked as to form a **single inventive concept**, i.e. having at least one common technical feature defining a contribution over the known prior art.

The IPEA agrees with the ISA advices that the present application lacks unity and identifies the following groups of inventions in the international application :

1. Claims : 1-27

   Methods for preventing proliferation of a tumor or for preventing tumor cell metastasis, comprising exposing a tumor cell to heparinase III, either native or modified.

   Methods for preparing therapeutic agents, i.e. HLGAG fragments, for tumor treatment comprising isolating of a portion of a tumor, treating it with heparinase III to produce HLGAG fragments, and isolating HLGAG fragments, possibly further comprising determining the sequence of the HLGAG fragments.

   Methods for treating a subject having a tumor, comprising administering to the subject a therapeutic, synthetic or isolated HLGAG fragment, identified or produced when the tumor is contacted with heparinase III.

   Pharmaceutical compositions comprising a therapeutic HLGAG fragment for preventing metastasis of a tumor cell, e.g. with an anti-cancer compound.

2. Claims : 28-49

A substantially pure heparinase III comprising a polypeptide according to SEQ ID NO:2, or having conservative substitutions thereof within residues non-essential to enzymatic function wherein at least one His residue from the group His36, His105, His110, His139, His152, His225, His234, His241, His424, His469 and His539 has been substituted with an Ala, Ser, Tyr, Thr or Lys residue.

A substantially pure heparinase III having a modified product profile which is at least 10% different than the product profile of native heparinase III.

A substantially pure heparinase III that can cleave a heparan sulfate having a modified k-cat value which is at least 10% different than a k-cat value of native heparinase III.

A pharmaceutical preparation comprising heparinase III as said.

An immobilized substantially pure modified heparinase III comprising a modified heparinase III as said and a solid support.

A method of specifically cleaving a HLGAG comprising contacting a HLGAG with a modified heparinase III as said. e.g. wherein the heparinase III is administered to subject in need for inhibiting angiogenesis, or wherein the heparinase is administered to a tumor, or wherein the heparinase III is administered in a polymeric delivery device or in a vehicle for injection r in a vehicle for topical application (e.g. to the eye), or wherein the method is a method for sequencing HLGAG fragments.

3. Claims : 50-54

Methods for treating or preventing a subject having a cancer or at risk of developing a cancer, comprising administering a therapeutic HLGAG fragment, e.g. a composition of HLGAG fragments wherein at least 50%, 75% or 90% f the HLGAG fragments are di- or tri-sulfated disaccharides, or wherein the therapeutic HLGAG fragment is free of mono- or unsulfated disaccharides.

4. Claims : 55-60

A method for preparing LMWH comprising contacting an HLGAG sample with a modified heparinase III molecule to produce LMWH.

A composition comprising LMWH produced by contacting an HLGAG sample with a modified heparinase III. Methods of treating or preventing, e.g. of a disorder associated with coagulation, or of a tumor, or of psoriasis, or of neovascularization comprising administering to a subject a composition as said.

The prior art contains the following documents :
- WO9513830 disclosing and claiming the inhibitory effect of heparinase on angiogenesis, e.g. in a tumor.
- WO9201003 disclosing and claiming glycosaminoglycan derivatives and their use, e.g. in impeding the formation of tumor metastases.
- R. Godavarti et al. (1996) Biochem. Biophys. Res. Commun. 225 : 751-758 and WO9412618 disclosing heparinase III and its encoding gene from Flavobacterium heparinum
- EP0244236 and EP0394971 disclosing the preparation of a low-molecular weight heparin (LMWH) by chemical or enzymatic degradation of heparin or heparan sulfate, e.g. with the help of heparinase, and its use in inhibition of angiogenesis and the treatment of tumors.

In the light of these prior art documents, a first problem underlying this application can be defined as the need for further means and methods for preventing proliferation of a tumor or for preventing tumor cell metastasis. The solution as disclosed and claimed can be summarized as the provision of such means and methods comprising compositions containing heparinase III, or therapeutic heparin-like glycosaminoglycan (HLGAG) fragments obtained with the help of heparinase III and methods for the preparation and sequencing of these HLGAG fragments comprising the use of heparinase III, as well as the use of these compositions.

A second problem underlying the current application in view of the prior art can b summarized as the need for further heparinases. The solution as disclosed and claimed can be summarized as the need for further heparinases. The solution as disclosed and

claimed can be summarized as the provision of native or modified forms (mutants) of heparinase III, and the uses of these modified heparinases III.

A third problem underlying the current application in view of the prior art can be summarized as the need for further methods for the preparation of LMWH. The solution as disclosed and claimed can be summarized as the provision of a method for preparing LMWH comprising the use of a modified heparinase III, as well as the use of said LMWH in the preparation of pharmaceutical compositions and their use in treating or preventing disorders and diseases.

In view of the fact that methods for treating or preventing tumor proliferation or metatastasis, e.g. comprising administering heparinases, glycosaminoglycans or LMWH are known, and methods for their preparation are known, in view of the different problems underlying the different solutions as disclosed and claimed, and due to the fact that no other technical feature can be distinguished which, in the light of the prior art, could be regarded as special technical feature common to these solutions, the IPEA agrees with the ISA advices that there is no single inventive concept underlying the plurality of claimed inventions of the present application in the sense of Rule 13.1 PCT. Consequently there is a lack of unity and the groups mentioned above represent independent inventions.

The attention of the applicant is drawn to the fact that claims 1-27 further lack unity for the following reasons :

1. Claims 1-17 refer to methods for preventing tumor cell proliferation or metastasis by treating said cells with heparinase III.

2. Claims 18-19 refer to a method for preparing therapeutic HLGAG fragments for the treatment of a tumor by treating a portion of a tumor with heparinase III.

3. Claims 20-22 refer to methods of treatment of a subject having a tumor by administering a therapeutic HLGAG fragment to said subject.

4. Claims 23-27 refer to compositions comprising a heparinase III and a targeting molecule or a therapeutic HLGAG fragment.

The use of heparinase III (optionally linked to a targeting molecule) for preventing cancer being well known from documents D1 and D4 (see points V-3.1 and V-4 of the present opinion) and HLGAG fragments produced by heparinase III being well-known from documents D2 and D3 and the targeting (see points V-3.1 and V-3.2 of the present opinion), the IPEA fails to see what could be considered as an inventive common concept linking the different groups mentioned above. Therefore, the present application lacks unity and the different groups mentioned above represent independent inventions.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present application refers to methods for preventing proliferation of a tumor or for preventing tumor cell metastasis comprising exposing a tumor cell to an effective amount of (optionally modified) heparinase III, optionally in association with additional anti-cancer drugs. The application also refers to a method for preparing therapeutic agents (HLGAG fragments) for the treatment of a tumor and to compositions comprising heparinase III or therapeutic HLGAG fragments and a targeting molecule for targeting heparinase III to the tumor.

2. **Reference is made to the following documents :**

D1: WO 96 01648 A (IBEX TECHNOLOGIES R AND D INC ;ZIMMERMANN JOSEPH (US); VLODAVSKY I) 25 January 1996 (1996-01-25)

D2: WO 94 21689 A (CANCER RES CAMPAIGN TECH ;LYON MALCOLM (GB); GALLAGHER JOHN THOMAS) 29 September 1994 (1994-09-29)

D3: WO 93 19096 A (CANCER RES CAMPAIGN TECH) 30 September 1993 (1993-09-30)

D4: WO 95 13830 A (MASSACHUSETTS INST TECHNOLOGY ;CHILDRENS MEDICAL CENTER (US)) 26 May 1995 (1995-05-26)

3. The amendments filed with the letter of 11.06.02 are allowable under articles 19(2) and 34(2)(b) PCT.
The comments filed by the applicant with the letter of 11.07.02 have been taken

into account for drafting the present communication.

4. Lack of novelty; article 33(2) PCT.

4.1 The document D1 discloses, inter alia, the fact that selectively removing heparan sulphate from cell surfaces while leaving the extracellular matrix intact inhibits cell proliferation by down regulating the cell's response to growth factors and that this can be achieved by targeting heparin or heparan sulphate degrading activities to the cell surface. The glycosaminoglycan degrading enzymes comprise heparinase I, II and III. The targeting of said enzymes can be achieved by **genetically engineering a ligand binding functionality** into heparinase proteins or by physically controlling the localized enzyme concentration through the method of administration. (Abstract). Means of administration are disclosed on p.32 and include topically administration or injection.
Claim 8 discloses a method to diminish the response of a cell to growth factors by directing a glycosaminoglycan degrading enzyme to the surface of targeted cells. Claim 9 specifies that targeting is achieved by incorporating a cell specific ligand binding function and heparin or heparan sulfate degrading activity into a **fusion protein** having glycosaminoglycan degrading enzyme activity and claim 11 specifies that the ligand is specific to or present in higher concentration in **cancer cells** as compared with normal cells. Claims 17-28 refer to pharmaceutical compositions comprising a glycosaminoglycan degrading enzyme in combination with a pharmaceutically acceptable carrier and, optionally, means for targeting the enzyme to cells or tissues (claims 23- 25).

Even if D1 principally deals with the use of heparinases for stimulating cell proliferation, D1 also discloses and claims the use of heparinases to inhibit cell proliferation by targeting said heparinase to the cell surface. The IPEA considers that the choice of an appropriate cell specific ligand binding function and its incorporation into a fusion protein would have been easily achieved by the skilled person.

Moreover, even if the mechanism of action of the heparinase III was not

known from the authors of D1, the method disclosed in D1 is the same as the method disclosed in the present application, i.e. targeting a heparinase - which can be heparinase III - to a tumor cell. Since no further indications are given in the methods of the present application, the IPEA assumes that the mere presence of heparinase III at the surface of a tumor cell will be sufficient to prevent proliferation of the tumor cell or metastasis.
Moreover, the IPEA considers that the fusion protein disclosed in D1 can be considered as a modified heparinase III.

Therefore, the subject-matter of claims 1-5, 9, 11-14 and 16-17 can not be considered as novel in the sense of article 33(2) PCT.

Since D1 claims compositions comprising a heparinase or a fusion protein between a heparinase and a targeting molecule consisting of a hormone, an antibody or an integrin, the subject-matter of claims 23-26 can not be considered as novel in the sense of article 33(2) PCT.

4.2 The subject-matter of claims 6-8, 10, 15, 18-22 and 27 has never been disclosed in the documents cited in the International Search Report (ISR). Therefore, claims 6-8, 10, 15, 18-22 and 27 are considered as novel in the sense of article 33(2) PCT.

5. Lack of inventive step; article 33(3) PCT.

5.1 The IPEA considers that the skilled person, knowing from the document D1 that tumor growth or metastasis could be prevented by targeting a heparinase (including heparinase III) to the surface of a tumor cell, would have contemplated to administrate the heparinase in conjunction with other well-known anti-cancer compounds.
Therefore, claims 8 and 15 can not be considered as inventive (article 33(3) PCT).

The IPEA is also the opinion that the treatment of a tumor with heparinase in vitro can also not be considered as inventive. Therefore, claims 7 lacks inventive step in the sense of article 33(3) PCT.

The selection of specific tumor to be treated can also not be considered as inventive as long as this selection is not motivated by a technical purpose. For the moment, the IPEA fails to see such a technical purpose for the selection of the prostate tumor and melanoma. Therefore, claim 10 can not be considered as inventive (article 33(3) PCT).

The attention of the applicant is also drawn to the fact that the document D4 deals with the use of heparinases - including heparinase III - for inhibiting angiogenesis. An application of the methods disclosed in D4 is the treatment of solid tumors. Different ways of administration of heparinase are discussed, including direct injection in tumors (p. 38-39).
Even if the purpose for administering heparinase III in D4 (inhibiting angiogenesis which is necessary for solid tumor growth and metastasis) is not the same as in the present application, the administration of said heparinase to tumor cells for treating cancer has been suggested in D4. Therefore, the subject-matter of claims 1-17 can not be considered as inventive over the teaching of D4 (article 33(3) PCT).

5.2 The document D3 discloses oligosaccharides having growth factor binding affinity. These oligosaccharides can be prepared from glycosaminoglycans such as heparan sulphate and can be used either to activate and stimulate FGF activity or inhibit FGF activity. The use of said oligosaccharides for therapeutic purposes in medicine is also disclosed (Abstract). Some of the glycosaminoglycans were depolymerised using heparinitase (i.e. heparinase III) (p. 33-34, Depolymerisation of HS to selectively produce sulphated oligosaccharides). Further purifications steps are disclosed (p. 34-38). The use of the oligosaccharides for **antitumour treatment** is suggested (p. 40, lines 19-21). Claim 11 discloses an oligosaccharide product obtained by digestion of heparan sulphate by heparinitase, claim 25 discloses a method for obtaining such an oligosaccharide product using heparinitase, claims 33-35 refer to the therapeutical use of such an oligosaccharide product as an active FGF-activity inhibiting agent for controlling or reducing cell growth or proliferation, inter alia **for inhibiting cancer cell growth and metastasis** using an oligosaccharide product according to D3.

The IPEA considers that, knowing from D3 that oligosaccharides obtained by heparinase III digestion of heparan sulphate could be used for reducing cell growth, inter alia for inhibiting cancer cell growth and metastasis, would need no inventive activity to consider linking said fragments to well-known targeting molecules in order to target said compounds to a cancer cell.

Therefore, the subject-matter of claims 20 and 23-27 can not be considered as inventive in the sense of article 33(3) PCT.

5.3 The subject-matter of claims 18-19 and 21-22 has never been disclosed or suggested in the documents cited in the ISR. Therefore, claims 18-19 and 21-22 are considered as inventive in the sense of article 33(3) PCT.

6. Industrial applicability; article 33(4) PCT.

Claims 4-6, 9, 11, 15 and 20-22 refer to method of treatment of the human or animal body.
The methods of claims 1-3, 8, 10, 12-14 and 16 (partially) can be considered as method of treatment of the human or animal body as long as they are practised in vivo.
For the assessment of the present claims 4-6, 9, 11, 15 and 20-23 (completely) and claims 1-3, 8, 10, 12-14 and 16 (partially) on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

7. Further remarks concerning the application.

7.1 The attention of the applicant is drawn to the fact that, in the light of the prior art, the composition of the HLGAGs at the surface of a cell can greatly vary from one cell type to another. Therefore, the IPEA considers that it is questionable if the HLGAG fragments resulting from heparinase III treatment

of every type of tumor cell will result in the generation of HLGAG fragments having a tumor- or metastasis-preventing activity according to the present application.

7.2 Claims 23-27 refer to compositions comprising, inter alia, HLGAG fragments. The IPEA is the opinion that said claims are not clear for the following reasons :

(i) All heparinase III-generated HLGAG fragments will not have an effect for preventing tumor growth or metastasis. The IPEA considers that, faced to all the possible HLGAG fragments which could be generated by heparinase III treatment of a caner cell, the skilled will not be able to determine those having a tumor growth or metastasis-preventing activity without undue burden of experimentation.

(ii) The attention of the applicant is drawn to the fact that the HLGAG fragments, being products, should be defined in terms of technical features of the product. The IPEA is the opinion that the definition of the HLGAG fragments in term of process steps would also not be considered as sufficiently clear since the method for producing HLGAG fragments in the present application will give very different HLGAG fragments, depending on the nature of the HLGAG at the surface of the cell and the conditions of the heparinase III treatment.

20. A method for treating a subject having a tumor, comprising, administering to the subject a therapeutic HLGAG fragment to treat the tumor.

21. The method of 20, wherein the therapeutic HLGAG fragment administered to the subject is a synthetic HLGAG fragment generated based on the sequence of the HLGAG fragment identified when the tumor is contacted with heparinase III.

22. The method of 20, wherein the therapeutic HLGAG fragment administered to the subject is an isolated HLGAG fragment produced when the tumor is contacted with heparinase III.

23. A composition, comprising:

heparinase III or a therapeutic HLGAG fragment in an effective amount for preventing metastasis of a tumor cell and a targeting molecule for targeting the heparinase III to the tumor, in a pharmaceutically acceptable carrier, wherein the heparinase III or therapeutic HLGAG fragment is linked to the targeting molecule.

24. The composition of claim 23, wherein the heparinase III is a modified heparinase III.

25. The composition of claim 23, wherein the heparinase III is a native heparinase III.

26. The composition of claim 23, wherein the targeting molecule is a compound which binds specifically to an antigen on the surface of a tumor cell.

27. A composition, comprising:

heparinase III or a therapeutic HLGAG fragment in an effective amount for preventing metastasis of a tumor cell and an anti-cancer compound in a pharmaceutically acceptable carrier.

28. A substantially pure heparinase III, comprising:

TENT COOPERATION TREATY

IFD

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

INVITATION TO PAY ADDITIONAL FEES

(PCT Article 17(3)(a) and Rule 40.1)

To:
WOLF, GREENFIELD & SACKS, P.C.
Attn. LOCKHART, HELEN C.
600 Atlantic Avenue
Federal Reserve Plaza
Boston, Massachusetts 02210
UNITED STATES OF AMERICA

REGISTERED

Date of mailing *(day/month/year)* 01/02/2000	
Applicant's or agent's file reference M0656/7046WO	**PAYMENT DUE** within 45 ~~months~~/days from the above date of mailing
International application No. PCT/US 99/19841	International filing date *(day/month/year)* 27/08/1999

Applicant

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

1. This International Searching Authority

(i) considers that there are 2 *(number of)* inventions claimed in the international application covered by the claims indicated ~~below~~/on the extra sheet:

and it considers that the international application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated ~~below~~/on the extra sheet:

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(ii) [X] has carried out a partial international search (see Annex) [ ] will establish the international search report on those parts of the international application which relate to the invention first mentioned in claims Nos.:

1-21,31,46-57(compl.), 30,33-45 (partly)

(iii) will establish the international search report on the other parts of the international application only if, and to the extent to which, additional fees are paid

2. The applicant is hereby **invited,** within the time limit indicated above, to pay the amount indicated below:

EUR 945,00	x	1	=	EUR 945,00
Fee per additional invention		number of additional inventions		total amount of additional fees

Or, _____ x _____ = _____

The applicant is informed that, according to Rule 40.2(c), **the payment of any additional fee may be made under protest,** i.e., a reasoned statement to the effect that the international application complies with the requirement of unity of invention or that the amount of the required additional fee is excessive.

3. [X] Claim(s) Nos. see remark have been found to be unsearchable under Article 17(2)(b) because of defects under Article 17(2)(a) and therefore have not been included with any invention.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mireille Claudepierre

Form PCT/ISA/206 (July 1992)

INVITATION TO PAY ADDITIONAL FEES

International application No.
PCT/US 99/19841

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-21,31,46-57 (all completely), 30, 33-45 (all partly)

A modified heparinase II, pharmaceutical preparation comprising it, method of cleaving a glycosaminoglycan with said heparinase II, nucleic acid encoding it, end vector and host cell comprising said nucleic acid.

2. Claims: 22-29, 32 (all completely), 30, 33-45 (all partly)

A modified heparinase I having enzymatic activity that is not dependent on the presence of calcium, pharmaceutical preparation comprising said heparinase I, method of cleaving a glycosaminoglycan employing said heparinase I

MOTIVATION:

The closest prior art with respect to the present patent application is represented by The Journal of Biological Chemistry 273, pages 10160-10167 and Biochemistry 34, pages 14441-14448. The first document discloses heparinase II mutants with reduced enzymic activity toward heparin and/or heparan sulfate (see Table I), the latter document discloses heparinase I mutants also with reduced activity (see Table 3).

In the light of this prior art, the problem underlying the present application is the provision of further heparinase mutants with reduced activity,for use as a therapeutic agent. The solutions to this problem disclosed and claimed in the present application consists of amodified heparinase II with amodified product profile and a modified heparinase I having enzymatic activity that is not dependent on the presence of calcium.

Due to the fact that modified heparinases are known, and due to the essential differences in primary structure of heparinase II and heparinase I, and due to the fact that no other technical features can be distinguished which, in the light of the prior art could be regarded as special technical features in the sense of Rule 13.2 PCT, the ISA is of the opinion that there is no single inventive concept underlying the claimed inventions of the present application in the sense of Rule 13.1 PCT. Consequently there is lack of unity and the different inventions, are formulated as the different subjects mentioned above.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 206

Continuation of Box 3.

Although claims 35-44 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the heparinase.

Annex to Form PCT/ISA/206
COMMUNICATION RELATING TO THE RESULTS OF THE PARTIAL INTERNATIONAL SEARCH

International Application No
PCT/US 99/19841

1. The present communication is an Annex to the invitation to pay additional fees (Form PCT/ISA/206). It shows the results of the international search established on the parts of the international application which relate to the invention first mentioned in claims Nos.:
see 'Invitation to pay additional fees'
2. This communication is not the international search report which will be established according to Article 18 and Rule 43.

3. If the applicant does not pay any additional search fees, the information appearing in this communication will be considered as the result of the international search and will be included as such in the international search report.

4. If the applicant pays additional fees, the international search report will contain both the information appearing in this communication and the results of the international search on other parts of the international application for which such fees will have been paid.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 34635 A (IBEX TECHNOLOGIES ;ZIMMERMANN JOSEPH (US)) 21 December 1995 (1995-12-21) page 32, line 1 - line 6; example 7 ---	1-21,30, 31,33, 34,46-57
X	SHRIVER Z ET AL.: "Heparinase II from flavobacterium heparinum: Role of histidine residues in enzymatic activity as probed by chemical modification and site-directed mutagenesis" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 17, 24 April 1998 (1998-04-24), pages 10160-10167, XP002127285 MD US table I ---	1-5, 7-13,16, 17, 19-21, 30,31, 33,34, 46-52
A	SASISEKHARAN R ET AL: "HEPARINASE I FROM FLAVOBACTERIUM HEPARINUM: THE ROLE OF THE CYSTEINE RESIDUE IN CATALYSIS AS PROBED BY CHEMICAL MODIFICATION AND SITE-DIRECTED MUTAGENESIS" BIOCHEMISTRY,US,AMERICAN CHEMICAL SOCIETY. EASTON, PA, vol. 34, no. 44, page 14441-14448 XP002026383 ISSN: 0006-2960 cited in the application the whole document --- -/--	1-21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

3

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	SHRIVER Z ET AL.: "Heparinase II from Flavobacterium heparinum: Role of cysteine in enzymatic activity as probed by chemical modification and site-directed mutagenesis" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 36, 4 September 1998 (1998-09-04), pages 22904-22912, XP002127286 MD US the whole document	1-21,30, 31,33, 34,46-52

3

Patent Family Annex

In[illegible]ion on patent family members

International Application No
[illegible]/US 99/19841

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9534635 A	21-12-1995	US 5681733 A	28-10-1997
		AU 694241 B	16-07-1998
		AU 2771095 A	05-01-1996
		CA 2192159 A	21-12-1995
		EP 0763101 A	19-03-1997
		JP 10511841 T	17-11-1998
		US 5919693 A	06-07-1999
		ZA 9504781 A	08-02-1996

PATENT COOPERATION TREATY

IFD

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
WOLF, GREENFIELD & SACKS, P.C.
Attn. LOCKHART, HELEN C.
600 Atlantic Avenue
Federal Reserve Plaza
Boston, Massachusetts 02210
UNITED STATES OF AMERICA

Applicant's or agent's file reference M0656/7046WO	Date of mailing *(day/month/year)* 27/04/2000
International application No. PCT/US 99/ 19841	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
Applicant MASSACHUSETTS INSTITUTE OF TECHNOLOGY	International filing date *(day/month/year)* 27/08/1999

DOCKETED
MAY 3 2000

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

	Initials
File Folder	☑
ECB	☐
Docket Entry	☑
Docket Cross Off	☑
Order Copies	☐
Annuities	☐
Confirmation	☐

06/21/00

2. ☐ The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Andria Overbeeke-Siepkes

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
M0656/7046WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/ 19841	27/08/1999	27/08/1998
Applicant		
MASSACHUSETTS INSTITUTE OF TECHNOLOGY		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readble form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 2

☒ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☐ None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/19841

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 35-44 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the heparinase.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Claims 1-21,31,46-57,(all completely) 30,33-45 (all partially)

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-21,31,46-57 (all completely), 30, 33-45 (all partly)

A modified heparinase II, pharmaceutical preparation comprising it, method of cleaving a glycosaminoglycan with said heparinase II, nucleic acid encoding it, end vector and host cell comprising said nucleic acid.

2. Claims: 22-29, 32 (all completely), 30, 33-45 (all partly)

A modified heparinase I having enzymatic activity that is not dependent on the presence of calcium, pharmaceutical preparation comprising said heparinase I, method of cleaving a glycosaminoglycan employing said heparinase I

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/19841

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/60 C12N9/88 A61K38/51 C12N11/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 34635 A (IBEX TECHNOLOGIES ;ZIMMERMANN JOSEPH (US)) 21 December 1995 (1995-12-21) page 32, line 1 - line 6; example 7 ---	1-21,30, 31,33, 34,46-57
X	SHRIVER Z ET AL.: "Heparinase II from flavobacterium heparinum: Role of histidine residues in enzymatic activity as probed by chemical modification and site-directed mutagenesis" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 17, 24 April 1998 (1998-04-24), pages 10160-10167, XP002127285 MD US table I --- -/--	1-5, 7-13,16, 17, 19-21, 30,31, 33,34, 46-52

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 January 2000	27.04.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	CUPIDO, M

3

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SASISEKHARAN R ET AL: "HEPARINASE I FROM FLAVOBACTERIUM HEPARINUM: THE ROLE OF THE CYSTEINE RESIDUE IN CATALYSIS AS PROBED BY CHEMICAL MODIFICATION AND SITE-DIRECTED MUTAGENESIS" BIOCHEMISTRY,US,AMERICAN CHEMICAL SOCIETY. EASTON, PA, vol. 34, no. 44, page 14441-14448 XP002026383 ISSN: 0006-2960 cited in the application the whole document ---	1-21
X,P	SHRIVER Z ET AL.: "Heparinase II from Flavobacterium heparinum: Role of cysteine in enzymatic activity as probed by chemical modification and site-directed mutagenesis" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 36, 4 September 1998 (1998-09-04), pages 22904-22912, XP002127286 MD US the whole document -----	1-21,30, 31,33, 34,46-52

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/19841

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9534635 A	21-12-1995	US 5681733 A	28-10-1997
		AU 694241 B	16-07-1998
		AU 2771095 A	05-01-1996
		CA 2192159 A	21-12-1995
		EP 0763101 A	19-03-1997
		JP 10511841 T	17-11-1998
		US 5919693 A	06-07-1999
		ZA 9504781 A	08-02-1996

PATENT COOPERATION TREATY

TFT

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
LOCKHART, Helen C.
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210
ETATS-UNIS D'AMERIQUE

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	21.06.2000
Applicant's or agent's file reference: M0656/7046WO	**REPLY DUE** **within 3 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/19841	27/08/1999	27/08/1998

International Patent Classification (IPC) or both national classification and IPC
C12N15/60

Applicant
MASSACHUSETTS INSTITUTE OF TECHNOLOGY

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☒	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☒	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain document cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 27/12/2000.

Name and mailing address of the international preliminary examining authority:
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer / Examiner
Roscoe, R

Formalities officer (incl. extension of time limits)
Vullo, C
Telephone No. +49 89 2399 8061

I. Basis of the opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*):

Description, pages:

1-77 as originally filed

Claims, No.:

1-57 as originally filed

Drawings, sheets:

1/4-4/4 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

II. Priority

1. ☐ This opinion has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

I. Basis

The documents mentioned in the present written opinion / International Preliminary Examination Report are numbered as in the search report, i.e. D1 corresponds to the first document of the search report etc.

Sequence listing pages 1-8 are also included in the basis of the present assessment.

II. Priority

The entire subject-matter of the present application appears to be entitled to priority from US application number 60/098153 (08.10.99). Hence, document D4 is not relevant prior art.

III. No Opinion

No opinion can be given for those claims for which no International Search Report has been established (i.e. claims 22-29, 30(part), 32, (33-45)(part)).

IV. Lack of Unity

The present application comprises 2 invention groups as set out in the International Search Report. Preliminary Examination can only be carried out on invention group I - modified heparinase II and matter relating thereto (claims 1-21, 30(part), 31, (33-45)(part), 46-57).

Invention group I is further considered intrinsically non-unitary. Mutants of the kind claimed by applicant are specified in the prior art. Hence, no common inventive concept would appear to link the claimed mutants. Applicant will have to eventually limit himself to a specific mutant. However, for practical reasons, this matter shall not be addressed in the International Phase.

V. Reasoned statement on Novelty, Inventive Step and Industrial Applicability

- **Novelty (Art.33(2) PCT)**

D2 is the closest prior art. D2 discloses various Heparinase II mutants in which cysteine residues have been modified. Modifications of individual histidine residues at one of positions 48, 238, , 249, 252, 347, 440, 473, 579, 682, to alanine were tested. Several mutants were void of enzymatic activity to either heparin or heparin sulphate (238, 406, 408, 451, 579). Mutants 252, 347 and 440, however, displayed differential activity towards heparin or heparin sulphate. The H347A mutant showed a marked decrease in activity (suggested to be due to proximity to cysteine 348). H440A has a reduced activity towards heparin. Precise quantitative data relating to the activity of the different mutants is not given. The document does not suggest medical applications yet suggests that mutants may be useful in developing heparinase II as a biological tool.

Until proven otherwise by the applicant, D2 is cited as novelty-destroying against the following claims (applicant is also referred to section on clarity): 1-5, 8-13, 16, 17, 20, 21, 30, 31, 46, 47, 52, 55-57. Indeed certain of these claims specifically refer to products already disclosed in D2 (claims 13, 16, 17, 31, 52).

D1 discloses the sequence of Heparinase II and recombinant expression thereof (example 7). No mutants with modified activities are disclosed. Hence, D1 does not anticipate any of the present claims.

- **Inventive Step (Art.33(3) PCT)**

With regard to uses of Heparinases (including potential medical uses), it is noted that given the knowledge of the possible role of hesparinases and their substrates in various disease states (see for example introduction of D1), and further in view of the fact that applicant has not been adding to the medical knowledge in the field, claims to medical uses of heparinase mutants could only be considered inventive where the mutants are inventive per se. The same applies to trivial matter such as pharmaceutical compositions or immobilized enzymes.

Claims 14, 15 and 49 relate specifically to C348 mutants. D2 already implicates this position as a potential active site residue (p.10166, col.2). Hence, mutants at

this position would be obvious to make for the purpose of testing whether the implication is correct.

Since no inventive mutants are identified, inventive step is not acknowledged for any of the claims.

- **Industrial Applicability (Art.33(4) PCT)**

For the assessment of the present claims 30-32, 35-44, 46, 47, 49 and 50 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Claims 30-32, 35-44, 46, 47, 49 and 50 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

VIII. **Certain observations**

- **Clarity (Art.6 PCT)**

The term "product profile" is considered technically unclear.

The majority of the claims can be considered unclear since they relate to modified enzyme defined by the result to be achieved. Obtaining an enzyme with a somehow modified substrate specificity / reaction rate is the problem which applicant addresses with the claimed subject-matter. The claims need to specify the solution to the problem i.e. how the problem was overcome (in this case by introducing specific mutations). Further, claims need to clearly distinguish the claimed subject-matter from the prior art. In the present case, it is unclear which

of applicants mutations and which in the prior art (which overlaps substantially) can be considered to meet the specified "product profile" alterations. Novelty cannot be acknowledged for any of the claims where this is unclear. Indeed, given that applicants mutants were mainly known in the prior art and that it is to be assumed that the claims are drafted according to the types of effect achieved by these mutants, it would appear very likely that a substantial number of the claims are anticipated be the mutants already disclosed in D2..

3. Additional observations, if necessary:

   see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application,

☒ claims Nos. 22-29, 30(part), 32, (33-45)(part),

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 22-29, 30(part), 32, (33-45)(part).

IV. Lack of unity of invention

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with for the following reasons and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees:

   see separate sheet

3. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this opinion:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-21, 30(part), 31, (33-45)(part), 46-57 .

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-5, 8-13, 16, 17, 20, 21, 30, 31, 46, 47, 52, 55-57
Inventive step (IS)	Claims	1-21, 30, 31, 33-45, 46-57
Industrial applicability (IA)	Claims	30-32, 35-44, 46, 47, 49 and 50

2. Citations and explanations

   see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

EPA/EPO/OEB
D-80298 München
+49 89 2399-0
TX 523 656 epmu d
FAX +49 89 2399-4465

Correspondence with the EPO on PCT Chapter II demands

In order to ensure that your PCT Chapter II demand is dealt with as promptly as possible you are requested to use the enclosed self-adhesive labels with any correspondence relating to the demand sent to the Munich Office.

One of these labels should be affixed to a prominent place in the upper part of the letter or form etc. which you are filing.

PATENT COOPERATION TREATY

1 PD

DOCKETED
OCT 31 2000

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

LOCKHART, Helen C.
WOLF, GREENFIELD & SACKS,
600 Atlantic Avenue
Boston, Massachusetts 02210
ETATS-UNIS D'AMERIQUE

		Initials
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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	20.10.2000
Applicant's or agent's file reference M0656/7046WO	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/19841	27/08/1999	27/08/1998

Applicant
MASSACHUSETTS INSTITUTE OF TECHNOLOGY

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Vullo, C Tel.+49 89 2399-8061

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
M0656/7046WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/19841	27/08/1999	27/08/1998
International Patent Classification (IPC) or national classification and IPC C12N15/60		
Applicant MASSACHUSETTS INSTITUTE OF TECHNOLOGY		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21/03/2000	20.10.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Roscoe, R Telephone No. +49 89 2399 2554

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/19841

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-77 as originally filed

Claims, No.:

1-57 as originally filed

Drawings, sheets:

1/4-4/4 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 22-29, 30(part), 32, (33-45)(part).

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 22-29, 30(part), 32, (33-45)(part).

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-21, 30(part), 31, (33-45)(part), 46-57 .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	6, 7, 14, 15, 18, 19, 33-45(part), 48-51, 53, 54
	No:	Claims	1-5, 8-13, 16, 17, 20, 21, 30, 31, 46, 47, 52, 55-57
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-21, 30, 31, 33-45, 46-57
Industrial applicability (IA)	Yes:	Claims	1-21, 33, 34, 45, 48, 51-57
	No:	Claims	30-32, 35-44, 46, 47, 49 and 50

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

I. Basis

The documents mentioned in the present International Preliminary Examination Report are numbered as in the search report, i.e. D1 corresponds to the first document of the search report etc.

Sequence listing pages 1-8 are also included in the basis of the present assessment.

II. Priority

The entire subject-matter of the present application appears to be entitled to priority from US application number 60/098153 (08.10.99). Hence, document D4 is not relevant prior art.

III. No Opinion

No opinion can be given for those claims for which no International Search Report has been established (i.e. claims 22-29, 30(part), 32, (33-45)(part)).

IV. Lack of Unity

The present application comprises 2 invention groups as set out in the International Search Report. Preliminary Examination can only be carried out on invention group I - modified heparinase II and matter relating thereto (claims 1-21, 30(part), 31, (33-45)(part), 46-57).

Invention group I is further considered intrinsically non-unitary. Mutants of the kind claimed by applicant are specified in the prior art. Hence, no common inventive concept would appear to link the claimed mutants. Applicant will have to eventually limit himself to a specific mutant. However, for practical reasons, this matter shall not be addressed in the International Phase.

V. Reasoned statement on Novelty, Inventive Step and Industrial Applicability

- **Novelty (Art.33(2) PCT)**

D2 is the closest prior art. D2 discloses various Heparinase II mutants in which cysteine residues have been modified. Modifications of individual histidine residues at one of positions 48, 238, , 249, 252, 347, 440, 473, 579, 682, to alanine were tested. Several mutants were void of enzymatic activity to either heparin or heparin sulphate (238, 406, 408, 451, 579). Mutants 252, 347 and 440, however, displayed differential activity towards heparin or heparin sulphate. The H347A mutant showed a marked decrease in activity (suggested to be due to proximity to cysteine 348). H440A has a reduced activity towards heparin. Precise quantitative data relating to the activity of the different mutants is not given. The document does not suggest medical applications yet suggests that mutants may be useful in developing heparinase II as a biological tool.

Until proven otherwise by the applicant, D2 is cited as novelty-destroying against the following claims (applicant is also referred to section on clarity): 1-5, 8-13, 16, 17, 20, 21, 30, 31, 46, 47, 52, 55-57. Indeed certain of these claims specifically refer to products already disclosed in D2 (claims 13, 16, 17, 31, 52).

D1 discloses the sequence of Heparinase II and recombinant expression thereof (example 7). No mutants with modified activities are disclosed. Hence, D1 does not anticipate any of the present claims.

- **Inventive Step (Art.33(3) PCT)**

With regard to uses of Heparinases (including potential medical uses), it is noted that given the knowledge of the possible role of hesparinases and their substrates in various disease states (see for example introduction of D1), and further in view of the fact that applicant has not been adding to the medical knowledge in the field, claims to medical uses of heparinase mutants could only be considered inventive where the mutants are inventive per se. The same applies to trivial matter such as pharmaceutical compositions or immobilized enzymes.

Claims 14, 15 and 49 relate specifically to C348 mutants. D2 already implicates this position as a potential active site residue (p.10166, col.2). Hence, mutants at

this position would be obvious to make for the purpose of testing whether the implication is correct.

Since no inventive mutants are identified, inventive step is not acknowledged for any of the claims.

- **Industrial Applicability (Art.33(4) PCT)**

For the assessment of the present claims 30-32, 35-44, 46, 47, 49 and 50 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Claims 30-32, 35-44, 46, 47, 49 and 50 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

VIII. **Certain observations**

- **Clarity (Art.6 PCT)**

The term "product profile" is considered technically unclear.

The majority of the claims can be considered unclear since they relate to modified enzyme defined by the result to be achieved. Obtaining an enzyme with a somehow modified substrate specificity / reaction rate is the problem which applicant addresses with the claimed subject-matter. The claims need to specify the solution to the problem i.e. how the problem was overcome (in this case by introducing specific mutations). Further, claims need to clearly distinguish the claimed subject-matter from the prior art. In the present case, it is unclear which

of applicants mutations and which in the prior art (which overlaps substantially) can be considered to meet the specified "product profile" alterations. Novelty cannot be acknowledged for any of the claims where this is unclear. Indeed, given that applicants mutants were mainly known in the prior art and that it is to be assumed that the claims are drafted according to the types of effect achieved by these mutants, it would appear very likely that a substantial number of the claims are anticipated be the mutants already disclosed in D2.

PATENT COOPERATION TREATY

IFD

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

INVITATION TO PAY ADDITIONAL FEES

(PCT Article 17(3)(a) and Rule 40.1)

To:
WOLF, GREENFIELD & SACKS, P.C.
Attn. Lockhart, Helen C.
600 Atlantic Avenue
Boston, Massachusetts 02210
UNITED STATES OF AMERICA

REGISTERED

	Date of mailing *(day/month/year)* 26/10/2001
Applicant's or agent's file reference M0656/7063WO	**PAYMENT DUE** within 45 ~~months~~/days from the above date of mailing
International application No. PCT/US 01/07464	International filing date *(day/month/year)* 08/03/2001

Applicant

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

1. This International Searching Authority

(i) considers that there are 4 *(number of)* inventions claimed in the international application covered by the claims indicated ~~below~~/on the extra sheet:

Subject to PTA? YES/NO
per docket/ECB
Smt 11-6-01

and it considers that the international application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated ~~below~~/on the extra sheet:

DOCKETED
NOV 07 2001

	Initials
File Folder 12-5-01	
ECB 12-26-01	☑
Docket Entry 12-10-01	☑
Docket Cross Off	☐
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Annuities	☐
Confirmation	☐

(ii) [X] has carried out a partial international search (see Annex) [ ] will establish the international search report on those parts of the international application which relate to the invention first mentioned in claims Nos.:
1-27

(iii) will establish the international search report on the other parts of the international application only if, and to the extent to which, additional fees are paid

2. The applicant is hereby **invited,** within the time limit indicated above, to pay the amount indicated below:

EUR 945,00	x	3	=	EUR 2.835,00
Fee per additional invention		number of additional inventions		total amount of additional fees

Or, _____ x _____ = _____

The applicant is informed that, according to Rule 40.2(c), **the payment of any additional fee may be made under protest,** i.e., a reasoned statement to the effect that the international application complies with the requirement of unity of invention or that the amount of the required additional fee is excessive.

3. [X] Claim(s) Nos. see remark have been found to be unsearchable under Article 17(2)(b) because of defects under Article 17(2)(a) and therefore have not been included with any invention.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mireille Claudepierre

Form PCT/ISA/206 (July 1992)

INVITATION TO PAY ADDITIONAL FEES

International application No.
PCT/US 01/07464

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-27

Methods for preventing proliferation of a tumor or for preventing tumor cell metastasis, comprising exposing a tumor cell to heparinase III, either native of modified.

Methods for preparing therapeutic agents, i.e., HLGAG fragments, for tumor treatment, comprising isolating of a portion of a tumor, treating it with heparinase III to produce HLGAG fragments, and isolating HLGAG fragments, possibly further comprising determining the sequence of the HLGAG fragments.

Methods for treating a subject having a tumor, comprising administering to the subject a therapeutic, synthetic or isolated HLGAG fragment, identified or produced when the tumor is contacted with heparinase III.

Pharmaceutical compositions comprising heparinase III, either native or modified, for preventing metastasis of a tumor cell, e.g., with a targeting molecule for targeting heparinase III to the tumor, the targeting molecule being a compound which binds specifically to an antigen on the surface of a tumor cell, or with an anti-cancer compound.

Pharmaceutical compositions comprising a therapeutic HLGAG fragment for preventing metastasis of a tumor cell, e.g., with an anti-cancer compound.

2. Claims: 28-49

A substantially pure heparinase III comprising a polypeptide according to SEQ ID NO:2, or having conservative substitutions thereof within residues non-essential to enzymatic function, wherein at least on His residue from the group of His36, His105, His110, His 139, His152, His 225, His234, His241, His424, His469 and His539 has been substituted with an Ala, Ser, Tyr, Thr or Lys residue.

A substantially pure heparinase III having a modified product profile which is at least 10% different than the product profile of native heparinase III.

A substantially pure heparinase III that can cleave a heparan sulfate having a modified k-cat value which is at least 10% different than a k-cat value of native heparinase III.

A pharmaceutical preparation comprising a heparinase III as said.

An immobilized substantially pure modified heparinase III

comprising a modified heparinase III as said and a solid support.

A method of specifically cleaving a HLGAG comprising contacting a HLGAG with a modified heparinase III as said, e.g., wherein the heparinase III is administered to subject in need for inhibiting angiogenesis, or wherein the heparinase is administered to a tumor, or wherein the heparinase III is adminstered in a polymeric delivery device or in a vehicle for injection or in a vehicle for topical application (e.g., to the eye), or wherein the method is a method for sequencing HLGAG fragments.

3. Claims: 50-54

Methods for treating or preventing a subject having a cancer or at risk of developing a cancer, comprising administering a therapeutic HLGAG fragment, e.g., a composition of HLGAG fragments wherein at least 50%, 75% or 90% of the HLGAG fragments are di- or tri-sulfated disaccharides, or wherein the therapeutic HLGAG fragment is free of mono- or unsulfated disaccharides.

4. Claims: 55-60

A method for preparing LMWH comprising contacting an HLGAG sample with a modified heparinase III molecule to produce LMWH.

A composition comprising LMWH produced by contacting an HLGAG sample with a modified heparinase III. Methods of treating or preventing, e.g., of a disorder associated with coagulation, or of a tumor, or of psoriasis, or of neovascularization, comprising administering to a subject a composition as said.

Motivation of the Objection against Unity

The prior art contains the following documents:
- WO9513830, disclosing and claiming the inhibitory effect of heparinase on angiogenesis, e.g., in a tumor;
- WO9201003, disclosing and claiming glycosaminoglycan derivatives and their use, e.g., in impeding the formation of tumor metastases;
- R. Godavarti et al. (1996) Biochem. Biophys. Res. Commun. 225:751-758 and WO9412618, disclosing heparinase III and its encoding gene from Flavobacterium heparinum;
- EP0244236 and EP0394971, disclosing the preparation of a low-molecular weight heparin (LMWH) by chemical or enzymatic degradation of heparin or heparan sulphate, e.g., with the help of heparinase, and its use in inhibition of angiogenesis and the treatment of tumors.

In the light of these prior art documents, a first problem underlying this application can be defined as the need for further means and methods for preventing proliferation of a tumor or for preventing tumor

cell metastasis. The solution as disclosed and claimed can be summarized as the provision of such means and methods comprising compositions containing heparinase III, or therapeutic heparin-like glycosaminoglycan (HLGAG) fragments obtained with the help of heparinase III and methods for the preparation and sequencing of these HLGAG fragments comprising the use of heparinase III, as well as the use of these compositions.

A second problem underlying the current application in view of the prior art can be summarized as the need for further heparinases. The solution as disclosed and claimed can be summarized as the provision of native or modified forms (mutants) of heparinase III, and the uses of these modified heparinases III.

A third problem underlying the current application in view of the prior art can be summarized as the need for further methods of treating or preventing a subject having a cancer or at risk of developing cancer. The solution as disclosed and claimed can be summarized as the provision of such methods comprising administering a therapeutic HLGAG fragment, or a composition of therapeutic HLGAG fragments with a defined content of di- or tri-sulfated disaccharides.

A fourth problem underlying the current application can be summarized as the need for further methods for the preparation of LMWH. The solution as disclosed and claimed can be summarized as the provision of a method for preparing LMWH comprising the use of a modified heparinase III, as well as the use of said LMWH in the preparation of pharmaceutical compositions and their use in treating or preventing disorders and diseases.

In view of the fact that methods for treating or preventing tumor proliferation or metastasis, e.g., comprising administering heparinases, glycosaminoglycans or LMWH, are known, in view of the fact that heparinase III, glycosaminoglycans and LMWH are known, and methods for their preparation, are known, in view of the different problems underlying the different solutions as disclosed and claimed, and due to the fact that no other technical feature can be distinguished which, in the light of the prior art, could be regarded as special technical features common to these solutions, the ISA is of the opinion that there is no single inventive concept underlying the plurality of claimed inventions of the present application in the sense of Rule 13.1 PCT. Consequently there is a lack of unity and different inventions, not belonging to a common inventive concept are formulated as the different subjects on the communication pursuant to Art. 17(3)(a) PCT.

The application has been divided into the above (groups of) inventions which individually are considered to meet the requirement of unity. If additional fees are paid for (one or more of) the, as yet, unsearched invention(s), the subsequent search(es) might reveal prior art which leads to a finding of lack of unity a posteriori within (one or more of) the, as yet, unsearched invention(s). Should this be the case, as a rule, no further invitation to pay additional fees will be issued. Only the first identified invention in each group of inventions, for which additional search fees have been paid in due time and which subsequently is considered to lack unity, will be searched.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 206

Continuation of Box 3.

Although claims 1-22, insofar as in vivo methods are concerned, are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Anne. Form PCT/ISA/206
COMMUNICATION RELATING TO THE RESULTS OF THE PARTIAL INTERNATIONAL SEARCH

International Application No
PCT/US 01/07464

1. The present communication is an Annex to the invitation to pay additional fees (Form PCT/ISA/206). It shows the results of the international search established on the parts of the international application which relate to the invention first mentioned in claims Nos.:
1-27
2. This communication is not the international search report which will be established according to Article 18 and Rule 43.

3. If the applicant does not pay any additional search fees, the information appearing in this communication will be considered as the result of the international search and will be included as such in the international search report.

4. If the applicant pays additional fees, the international search report will contain both the information appearing in this communication and the results of the international search on other parts of the international application for which such fees will have been paid.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 01648 A (IBEX TECHNOLOGIES R AND D INC ;ZIMMERMANN JOSEPH (US); VLODAVSKY I) 25 January 1996 (1996-01-25) the whole document page 3, line 23 -page 4, line 5 page 9, line 17 -page 10, line 30 examples 3-5,9-16 claims 1-28 ---	1-27
X	NATKE B ET AL.: "Heparinase treatment of bovine smooth muscle cells inhibits fibroblast growth factor-2 binding to fibroblast growth factor receptor but not FGF-2 mediated cellular proliferation" ANGIOGENESIS , vol. 3, no. 3, 1999, pages 249-257, XP001030515 abstract ---	1-27
X	MURPHY P R ET AL.: "Basic fibroblast growth factor binding and processing by human glioma cells." MOLECULAR AND CELLULAR ENDOCRINOLOGY, vol. 114, no. 1-2, 1995, pages 193-203, XP001030507 ISSN: 0303-7207 abstract --- -/--	1-17, 23-27

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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International Application No
PCT/US 01/07464

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 13830 A (MASSACHUSETTS INST TECHNOLOGY ;CHILDRENS MEDICAL CENTER (US)) 26 May 1995 (1995-05-26) the whole document page 9, line 29 -page 10, line 33 page 13, line 7 -page 40, line 18 claims 1-21 ---	1-17, 23-27
X	WO 94 21689 A (CANCER RES CAMPAIGN TECH ;LYON MALCOLM (GB); GALLAGHER JOHN THOMAS) 29 September 1994 (1994-09-29) the whole document page 3, line 21 -page 10, line 17 table 1 claims 1-33 ---	18-27
X	WO 93 19096 A (CANCER RES CAMPAIGN TECH) 30 September 1993 (1993-09-30) the whole document page 7, line 32 -page 15, line 27 tables 1,2 claims 1-40 ---	18-27
X	WO 93 05167 A (CHILDRENS MEDICAL CENTER) 18 March 1993 (1993-03-18) page 3, line 1 -page 4, line 3 page 11, line 25 -page 16, line 33 figure 3 ---	18-27
A	WO 92 01003 A (UNIV TEXAS) 23 January 1992 (1992-01-23) the whole document ---	18-27
X A	PADERA R ET AL.: "FGF-2/fibroblast growth factor receptor/heparin-like glycosaminoglycan interactions: A compensation model for FGF-2 signaling." FASEB JOURNAL, vol. 13, no. 13, October 1999 (1999-10), pages 1677-1687, XP002179630 ISSN: 0892-6638 the whole document ---	18-27
A	VENKATARAMAN G: "Sequencing complex polysaccharides" SCIENCE, vol. 286, 15 October 1999 (1999-10-15), pages 537-542, XP002179570 cited in the application the whole document --- -/--	19

6

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GODAVARTI R ET AL.: "Heparinase III from Flavobacterium heparinum: Cloning and recombinant expression in Escherichia coli." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 225, no. 3, 1996, pages 751-758, XP002179572 ISSN: 0006-291X cited in the application the whole document abstract page 751, line 15 -page 752, line 2 page 757, line 42-50 ---	
A	WO 94 12618 A (MASSACHUSETTS INST TECHNOLOGY ;UNIV IOWA RES FOUND (US)) 9 June 1994 (1994-06-09) the whole document ---	
A	LINHARDT R J ET AL.: "Examination of the substrate specificity of heparin and heparan sulfate lyases" BIOCHEMISTRY, vol. 29, no. 10, 1990, pages 2611-2617, XP002028479 ISSN: 0006-2960 cited in the application abstract page 2616, left-hand column, line 30 -right-hand column, line 15 ---	
A	ERNST S ET AL.: "Enzymatic degradation of glycosaminoglycans." CRITICAL REVIEWS IN BIOCHEMISTRY AND MOLECULAR BIOLOGY, vol. 30, no. 5, 1995, pages 387-444, XP001030549 ISSN: 1040-9238 cited in the application abstract ---	
A	AMEER G A ET AL.: "A new approach to regional heparinization: Design and development of a novel immobilized heparinase device." BLOOD PURIFICATION. MEETING INFO.: THIRD INTERNATIONAL CONFERENCE ON CONTINUOUS RENAL REPLACEMENT THERAPIES SAN DIEGO, CALIFORNIA, USA MARCH 5-7, 1998 , vol. 16, no. 2, 5 March 1998 (1998-03-05), pages 107-108, XP001032809 abstract --- -/--	

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Annex Form PCT/ISA/206
COMMUNICATION RELATING TO THE RESULTS OF THE PARTIAL INTERNATIONAL SEARCH

International Application No
PCT/US 01/07464

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 244 236 A (NOVO INDUSTRI AS) 4 November 1987 (1987-11-04) the whole document	
A	EP 0 394 971 A (KABIVITRUM AB ;HARVARD COLLEGE (US)) 31 October 1990 (1990-10-31) the whole document	
T	BERRY D ET AL.: "Distinct heparan sulfate glycosaminoglycans are responsible for mediating fibroblast growth factor-2 biological activity through different fibroblast growth factor receptors" FASEB JOURNAL ON LINE, 6 April 2001 (2001-04-06), XP002179629 the whole document	18-27

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Patent Family Annex

Information on patent family members

International Application No
PCT/US 01/07464

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9601648 A	25-01-1996	US 5997863 A	07-12-1999
		AU 707007 B2	01-07-1999
		AU 3094995 A	09-02-1996
		CA 2194370 A1	25-01-1996
		EP 0769961 A1	02-05-1997
		JP 10506609 T	30-06-1998
		WO 9601648 A1	25-01-1996
WO 9513830 A	26-05-1995	CA 2176934 A1	26-05-1995
		EP 0726773 A1	21-08-1996
		JP 9508892 T	09-09-1997
		WO 9513830 A1	26-05-1995
		US 5567417 A	22-10-1996
WO 9421689 A	29-09-1994	AU 6287594 A	11-10-1994
		CA 2136531 A1	29-09-1994
		EP 0642533 A1	15-03-1995
		WO 9421689 A1	29-09-1994
		JP 7507596 T	24-08-1995
WO 9319096 A	30-09-1993	AU 3763293 A	21-10-1993
		CA 2132750 A1	30-09-1993
		EP 0632818 A1	11-01-1995
		WO 9319096 A1	30-09-1993
		GB 2265905 A ,B	13-10-1993
		JP 7505179 T	08-06-1995
WO 9305167 A	18-03-1993	AU 2561792 A	05-04-1993
		US 5486599 A	23-01-1996
		WO 9305167 A1	18-03-1993
WO 9201003 A	23-01-1992	US 5262403 A	16-11-1993
		AU 8306791 A	04-02-1992
		WO 9201003 A1	23-01-1992
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		CA 2150263 A1	09-06-1994
		EP 0670892 A1	13-09-1995
		JP 8505767 T	25-06-1996
		WO 9412618 A1	09-06-1994
		US 5569600 A	29-10-1996
EP 0244236 A	04-11-1987	AU 588102 B2	07-09-1989
		AU 7225587 A	05-11-1987
		CA 1334081 A1	24-01-1995
		DK 217187 A ,B,	31-10-1987
		EP 0244236 A2	04-11-1987
		FI 871910 A ,B,	31-10-1987
		JP 1835416 C	11-04-1994
		JP 5042919 B	30-06-1993
		JP 62283103 A	09-12-1987
		NO 871783 A ,B,	02-11-1987
		US 5106734 A	21-04-1992
EP 0394971 A	31-10-1990	AU 5445290 A	16-11-1990
		CA 2053883 A1	25-10-1990
		WO 9012580 A1	01-11-1990
		EP 0394971 A1	31-10-1990

Patent Family Annex

Information on patent family members

National Application No
PCT/US 01/07464

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0394971 A		HU 59828 A2	28-07-1992
		NO 914133 A	21-10-1991
		PT 93847 A	20-11-1990

File Folder	2/28/02	Initials
ECB	3/07/02	
Docket Entry	(2)	
Docket Cross Off		
Order Copies		
Annuities		
Confirmation		

PATENT COOPERATION TREATY

Subject to PTA? YES/NO
per docket/ECB
1/29/02

IFD

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
WOLF, GREENFIELD & SACKS, P.C.
Attn. Lockhart, Helen C.
600 Atlantic Avenue
Boston, Massachusetts 02210
UNITED STATES OF AMERICA

DOCKETED
FEB 4 2002

Date of mailing *(day/month/year)*	22/01/2002
Applicant's or agent's file reference: M0656/7063WO	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No.: PCT/US 01/07464	International filing date *(day/month/year)*: 08/03/2001
Applicant: MASSACHUSETTS INSTITUTE OF TECHNOLOGY	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Catherine Humbert

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
M0656/7063WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 01/07464	08/03/2001	08/03/2000
Applicant		
MASSACHUSETTS INSTITUTE OF TECHNOLOGY		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 8 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readble form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 8

☒ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☐ None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/07464

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-22, insofar as in vivo methods are concerned, are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-27

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-27

Methods for preventing proliferation of a tumor or for preventing tumor cell metastasis, comprising exposing a tumor cell to heparinase III, either native of modified.

Methods for preparing therapeutic agents, i.e., HLGAG fragments, for tumor treatment, comprising isolating of a portion of a tumor, treating it with heparinase III to produce HLGAG fragments, and isolating HLGAG fragments, possibly further comprising determining the sequence of the HLGAG fragments.

Methods for treating a subject having a tumor, comprising administering to the subject a therapeutic, synthetic or isolated HLGAG fragment, identified or produced when the tumor is contacted with heparinase III.

Pharmaceutical compositions comprising heparinase III, either native or modified, for preventing metastasis of a tumor cell, e.g., with a targeting molecule for targeting heparinase III to the tumor, the targeting molecule being a compound which binds specifically to an antigen on the surface of a tumor cell, or with an anti-cancer compound.

Pharmaceutical compositions comprising a therapeutic HLGAG fragment for preventing metastasis of a tumor cell, e.g., with an anti-cancer compound.

2. Claims: 28-49

A substantially pure heparinase III comprising a polypeptide according to SEQ ID NO:2, or having conservative substitutions thereof within residues non-essential to enzymatic function, wherein at least on His residue from the group of His36, His105, His110, His 139, His152, His 225, His234, His241, His424, His469 and His539 has been substituted with an Ala, Ser, Tyr, Thr or Lys residue.

A substantially pure heparinase III having a modified product profile which is at least 10% different than the product profile of native heparinase III.

A substantially pure heparinase III that can cleave a heparan sulfate having a modified k-cat value which is at least 10% different than a k-cat value of native heparinase III.

A pharmaceutical preparation comprising a heparinase III as said.

An immobilized substantially pure modified heparinase III comprising a modified heparinase III as said and a solid support.

A method of specifically cleaving a HLGAG comprising contacting a HLGAG with a modified heparinase III as said, e.g., wherein the heparinase III is administered to subject in need for inhibiting angiogenesis, or wherein the heparinase is administered to a tumor, or wherein the heparinase III is adminstered in a polymeric delivery device or in a vehicle for injection or in a vehicle for topical application (e.g., to the eye), or wherein the method is a method for sequencing HLGAG fragments.

3. Claims: 50-54

Methods for treating or preventing a subject having a cancer or at risk of developing a cancer, comprising administering a therapeutic HLGAG fragment, e.g., a composition of HLGAG fragments wherein at least 50%, 75% or 90% of the HLGAG fragments are di- or tri-sulfated disaccharides, or wherein the therapeutic HLGAG fragment is free of mono- or unsulfated disaccharides.

4. Claims: 55-60

A method for preparing LMWH comprising contacting an HLGAG sample with a modified heparinase III molecule to produce LMWH.

A composition comprising LMWH produced by contacting an HLGAG sample with a modified heparinase III. Methods of treating or preventing, e.g., of a disorder associated with coagulation, or of a tumor, or of psoriasis, or of neovascularization, comprising administering to a subject a composition as said.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/07464

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/60 C12N9/88 C12P19/26 A61K38/51 A61K31/715
C08B37/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12P A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 01648 A (IBEX TECHNOLOGIES R AND D INC ;ZIMMERMANN JOSEPH (US); VLODAVSKY I) 25 January 1996 (1996-01-25) the whole document page 3, line 23 -page 4, line 5 page 9, line 17 -page 10, line 30 examples 3-5,9-16 claims 1-28 ---	1-27
X	NATKE B ET AL.: "Heparinase treatment of bovine smooth muscle cells inhibits fibroblast growth factor-2 binding to fibroblast growth factor receptor but not FGF-2 mediated cellular proliferation" ANGIOGENESIS, vol. 3, no. 3, 1999, pages 249-257, XP001030515 abstract --- -/--	1-27

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 October 2001	22. 01. 2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	van de Kamp, M

6

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MURPHY P R ET AL.: "Basic fibroblast growth factor binding and processing by human glioma cells." MOLECULAR AND CELLULAR ENDOCRINOLOGY, vol. 114, no. 1-2, 1995, pages 193-203, XP001030507 ISSN: 0303-7207 abstract ---	1-17, 23-27
X	WO 95 13830 A (MASSACHUSETTS INST TECHNOLOGY ;CHILDRENS MEDICAL CENTER (US)) 26 May 1995 (1995-05-26) the whole document page 9, line 29 -page 10, line 33 page 13, line 7 -page 40, line 18 claims 1-21 ---	1-17, 23-27
X	WO 94 21689 A (CANCER RES CAMPAIGN TECH ;LYON MALCOLM (GB); GALLAGHER JOHN THOMAS) 29 September 1994 (1994-09-29) the whole document page 3, line 21 -page 10, line 17 table 1 claims 1-33 ---	18-27
X	WO 93 19096 A (CANCER RES CAMPAIGN TECH) 30 September 1993 (1993-09-30) the whole document page 7, line 32 -page 15, line 27 tables 1,2 claims 1-40 ---	18-27
X	WO 93 05167 A (CHILDRENS MEDICAL CENTER) 18 March 1993 (1993-03-18) page 3, line 1 -page 4, line 3 page 11, line 25 -page 16, line 33 figure 3 ---	18-27
A	WO 92 01003 A (UNIV TEXAS) 23 January 1992 (1992-01-23) the whole document ---	18-27
A	PADERA R ET AL.: "FGF-2/fibroblast growth factor receptor/heparin-like glycosaminoglycan interactions: A compensation model for FGF-2 signaling." FASEB JOURNAL, vol. 13, no. 13, October 1999 (1999-10), pages 1677-1687, XP002179630 ISSN: 0892-6638 the whole document ---	18-27

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6

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	VENKATARAMAN G: "Sequencing complex polysaccharides" SCIENCE, vol. 286, 15 October 1999 (1999-10-15), pages 537-542, XP002179570 cited in the application the whole document	19
A	GODAVARTI R ET AL.: "Heparinase III from Flavobacterium heparinum: Cloning and recombinant expression in Escherichia coli." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 225, no. 3, 1996, pages 751-758, XP002179572 ISSN: 0006-291X cited in the application the whole document abstract page 751, line 15 -page 752, line 2 page 757, line 42-50	
A	WO 94 12618 A (MASSACHUSETTS INST TECHNOLOGY ;UNIV IOWA RES FOUND (US)) 9 June 1994 (1994-06-09) the whole document	
A	LINHARDT R J ET AL.: "Examination of the substrate specificity of heparin and heparan sulfate lyases" BIOCHEMISTRY, vol. 29, no. 10, 1990, pages 2611-2617, XP002028479 ISSN: 0006-2960 cited in the application abstract page 2616, left-hand column, line 30 -right-hand column, line 15	
A	ERNST S ET AL.: "Enzymatic degradation of glycosaminoglycans." CRITICAL REVIEWS IN BIOCHEMISTRY AND MOLECULAR BIOLOGY, vol. 30, no. 5, 1995, pages 387-444, XP001030549 ISSN: 1040-9238 cited in the application abstract	

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International Application No
PCT/US 01/07464

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	AMEER G A ET AL.: "A new approach to regional heparinization: Design and development of a novel immobilized heparinase device." BLOOD PURIFICATION. MEETING INFO.: THIRD INTERNATIONAL CONFERENCE ON CONTINUOUS RENAL REPLACEMENT THERAPIES SAN DIEGO, CALIFORNIA, USA MARCH 5-7, 1998, vol. 16, no. 2, 5 March 1998 (1998-03-05), pages 107-108, XP001032809 abstract	
A	EP 0 244 236 A (NOVO INDUSTRI AS) 4 November 1987 (1987-11-04) the whole document	
A	EP 0 394 971 A (KABIVITRUM AB ;HARVARD COLLEGE (US)) 31 October 1990 (1990-10-31) the whole document	
T	BERRY D ET AL.: "Distinct heparan sulfate glycosaminoglycans are responsible for mediating fibroblast growth factor-2 biological activity through different fibroblast growth factor receptors" FASEB JOURNAL ON LINE, 6 April 2001 (2001-04-06), XP002179629 the whole document	18-27

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 01/07464

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9601648 A	25-01-1996	US 5997863 A	07-12-1999
		AU 707007 B2	01-07-1999
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		CA 2194370 A1	25-01-1996
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		JP 9508892 T	09-09-1997
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		EP 0670892 A1	13-09-1995
		JP 8505767 T	25-06-1996
		WO 9412618 A1	09-06-1994
		US 5569600 A	29-10-1996
EP 0244236 A	04-11-1987	AU 588102 B2	07-09-1989
		AU 7225587 A	05-11-1987
		CA 1334081 A1	24-01-1995
		DK 217187 A ,B,	31-10-1987
		EP 0244236 A2	04-11-1987
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		JP 1835416 C	11-04-1994
		JP 5042919 B	30-06-1993
		JP 62283103 A	09-12-1987
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		US 5106734 A	21-04-1992
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		CA 2053883 A1	25-10-1990
		WO 9012580 A1	01-11-1990
		EP 0394971 A1	31-10-1990

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 01/07464

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0394971 A		HU 59828 A2	28-07-1992
		NO 914133 A	21-10-1991
		PT 93847 A	20-11-1990

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

WRITTEN OPINION

(PCT Rule 66)

To:
Lockhart, Helen C.
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)*	27/02/2002
Applicant's or agent's file reference: M0656/7063WO	REPLY DUE: within 1 / 00 months/days from the above date of mailing
International application No.: PCT/US 01/ 07464	International filing date *(day/month/year)*: 08/03/2001
Priority date *(day/month/year)*: 08/03/2000	
International Patent Classification (IPC) or both national classification and IPC: C12N15/60	
Applicant: MASSACHUSETTS INSTITUTE OF TECHNOLOGY	

1. This written opinion is the first drawn up by this International Preliminary Examining Authority.
2. This opinion contains indications relating to the following items:

- I [X] Basis of the opinion
- II [ ] Priority
- III [X] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV [ ] Lack of unity of invention
- V [X] Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI [ ] Certain documents cited
- VII [ ] Certain defects in the international application
- VIII [ ] Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4*bis*. For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 08/07/2002.

Name and mailing address of the IPEA/
European Patent Office
D-80298 Munich
Tel. (+49-89) 2399-0, Tx: 523656 epmu d
Fax: (+49-89) 2399-4465

Authorized officer
Examiner

Formalities officer
(incl. extension of time limits)
Tel. (+49-89) 2399 2828

I. Basis of the opinion

1. The basis of this written opinion is the application as originally filed.

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

2. The question of whether the claimed invention appears to be novel, to involve an inventive step, or to be industrially applicable has not been and will not be the subject of the international preliminary examination (Article 34 (4) (a) (i) (ii) PCT; see also international search report) in respect of:

2.1 Applications having an unnecessary plurality of independent claims (generally not more than 1 independent claim in the same category is necessary; Article 6 PCT);

2.2 unsearched subject-matter (Article 17 (2) (a), Rule 66.1 (e) PCT), e.g.

2.2.1 claimed subject-matter under Rule 39.1 PCT,

2.2.2 applications where the description, the claims, or the drawings fail to comply with the prescribed requirements to such an extent that no meaningful search could have been carried out;

2.3 claimed subject-matter under Rule 67.1 PCT.

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability

3. To the extent that the international preliminary examination has been carried out (see item III above), the following is pointed out:

In light of the documents cited in the international search report, it is considered that the invention as claimed in at least one of the independant claims does not appear to meet the criteria mentioned in Article 33 (1) PCT, i.e. does not appear to be novel and/or to involve an inventive step.

4. If amendments are filed, the Applicant must comply with the requirements of Rule 66.8 PCT and indicate the basis in the originally filed application of the amendments made (Article 34 (2) (b) PCT) otherwise these amendments will not be taken into consideration for the establishment of international preliminary examination.
The attention of the applicant is drawn to the fact that if the application contains an unjustified plurality of independent claims no examination of any of the claims will be carried out.

PATENT COOPERATION TREATY

IFD

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

Subject to PTA? YES
per docket/ECB
05/13/02

To:

Lockhart, Helen C.
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210
ETATS-UNIS D'AMERIQUE

DOCKETED
MAY 15 2002

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Confirmation	☐

6-12-02

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	08.05.2002
Applicant's or agent's file reference M0656/7063WO	**REPLY DUE** **within 2 month(s)** ✓ from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US01/07464	08/03/2001	08/03/2000

International Patent Classification (IPC) or both national classification and IPC

C12N15/60

Applicant

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

1. This written opinion is the ~~first~~ second drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☒	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain document cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 08/07/2002. ✓

7 days
7/15/02

Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer / Examiner Mundel, C Formalities officer (incl. extension of time limits) Hingel, W Telephone No. +49 89 2399 8717

Form PCT/IPEA/408 (cover sheet) (January 1994)

I. Basis of the opinion

1. With regard to the **elements** of the international application (Replacement *sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed"*):

Description, pages:

1-69 as originally filed

Claims, No.:

1-60 as originally filed

Drawings, sheets:

1/17-17/17 as originally filed

Sequence listing part of the description, pages:

1-3, filed with the letter of 11.04.01

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 28-60,

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 28-60.

2. A written opinion cannot be drawn due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☒ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with for the following reasons and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees:
see separate sheet

3. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this opinion:

☐ all parts.

☒ the parts relating to claims Nos. 1-27.

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-5, 9, 11-14, 16-17 and 23-27 (NO)
Inventive step (IS)	Claims	1-17 and 23-27 (NO)
Industrial applicability (IA)	Claims	4-6, 9, 11, 15 and 20-22 (completely) and 1-3, 8, 10, 12-14 and 16 (partially) (see Citations and explanations)

2. Citations and explanations
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

A unity objection was raised by the International Search Authority (ISA). Since the applicant didn't pay additional search fees, only invention 1 (claims 1-27) has been searched and, therefore, examined.

Re Item IV

Lack of unity of invention

According to **Rule 13 PCT** an application must relate only to one invention or to a group of inventions so linked as to form a **single inventive concept**, i.e. having at least one common technical feature defining a contribution over the known prior art.

The IPEA agrees with the ISA advices that the present application lacks unity and identifies the following groups of inventions in the international application :

1. Claims : 1-27

   Methods for preventing proliferation of a tumor or for preventing tumor cell metastasis, comprising exposing a tumor cell to heparinase III, either native or modified.

   Methods for preparing therapeutic agents, i.e. HLGAG fragments, for tumor treatment comprising isolating of a portion of a tumor, treating it with heparinase III to produce HLGAG fragments, and isolating HLGAG fragments, possibly further comprising determining the sequence of the HLGAG fragments.

   Methods for treating a subject having a tumor, comprising administering to the subject a therapeutic, synthetic or isolated HLGAG fragment, identified or produced when the tumor is contacted with heparinase III.

   Pharmaceutical compositions comprising a therapeutic HLGAG fragment for preventing metastasis of a tumor cell, e.g. with an anti-cancer compound.

2. Claims : 28-49

A substantially pure heparinase III comprising a polypeptide according to SEQ ID NO:2, or having conservative substitutions thereof within residues non-essential to enzymatic function wherein at least one His residue from the group His36, His105, His110, His139, His152, His225, His234, His241, His424, His469 and His539 has been substituted with an Ala, Ser, Tyr, Thr or Lys residue.

A substantially pure heparinase III having a modified product profile which is at least 10% different than the product profile of native heparinase III.

A substantially pure heparinase III that can cleave a heparan sulfate having a modified k-cat value which is at least 10% different than a k-cat value of native heparinase III.

A pharmaceutical preparation comprising heparinase III as said.

An immobilized substantially pure modified heparinase III comprising a modified heparinase III as said and a solid support.

A method of specifically cleaving a HLGAG comprising contacting a HLGAG with a modified heparinase III as said. e.g. wherein the heparinase III is administered to subject in need for inhibiting angiogenesis, or wherein the heparinase is administered to a tumor, or wherein the heparinase III is administered in a polymeric delivery device or in a vehicle for injection r in a vehicle for topical application (e.g. to the eye), or wherein the method is a method for sequencing HLGAG fragments.

3. Claims : 50-54

Methods for treating or preventing a subject having a cancer or at risk of developing a cancer, comprising administering a therapeutic HLGAG fragment, e.g. a composition of HLGAG fragments wherein at least 50%, 75% or 90% f the HLGAG fragments are di- or tri-sulfated disaccharides, or wherein the therapeutic HLGAG fragment is free of mono- or unsulfated disaccharides.

4. Claims : 55-60

A method for preparing LMWH comprising contacting an HLGAG sample with a modified heparinase III molecule to produce LMWH.

A composition comprising LMWH produced by contacting an HLGAG sample with a modified heparinase III. Methods of treating or preventing, e.g. of a disorder associated with coagulation, or of a tumor, or of psoriasis, or of neovascularization comprising administering to a subject a composition as said.

The prior art contains the following documents :
- WO9513830 disclosing and claiming the inhibitory effect of heparinase on angiogenesis, e.g. in a tumor.
- WO9201003 disclosing and claiming glycosaminoglycan derivatives and their use, e.g. in impeding the formation of tumor metastases.
- R. Godavarti et al. (1996) Biochem. Biophys. Res. Commun. 225 : 751-758 and WO9412618 disclosing heparinase III and its encoding gene from Flavobacterium heparinum
- EP0244236 and EP0394971 disclosing the preparation of a low-molecular weight heparin (LMWH) by chemical or enzymatic degradation of heparin or heparan sulfate, e.g. with the help of heparinase, and its use in inhibition of angiogenesis and the treatment of tumors.

In the light of these prior art documents, a first problem underlying this application can be defined as the need for further means and methods for preventing proliferation of a tumor or for preventing tumor cell metastasis. The solution as disclosed and claimed can be summarized as the provision of such means and methods comprising compositions containing heparinase III, or therapeutic heparin-like glycosaminoglycan (HLGAG) fragments obtained with the help of heparinase III and methods for the preparation and sequencing of these HLGAG fragments comprising the use of heparinase III, as well as the use of these compositions.

A second problem underlying the current application in view of the prior art can b summarized as the need for further heparinases. The solution as disclosed and claimed can be summarized as the need for further heparinases. The solution as disclosed and

claimed can be summarized as the provision of native or modified forms (mutants) of heparinase III, and the uses of these modified heparinases III.

A third problem underlying the current application in view of the prior art can be summarized as the need for further methods for the preparation of LMWH. The solution as disclosed and claimed can be summarized as the provision of a method for preparing LMWH comprising the use of a modified heparinase III, as well as the use of said LMWH in the preparation of pharmaceutical compositions and their use in treating or preventing disorders and diseases.

In view of the fact that methods for treating or preventing tumor proliferation or metatastasis, e.g. comprising administering heparinases, glycosaminoglycans or LMWH are known, and methods for their preparation are known, in view of the different problems underlying the different solutions as disclosed and claimed, and due to the fact that no other technical feature can be distinguished which, in the light of the prior art, could be regarded as special technical feature common to these solutions, the IPEA agrees with the ISA advices that there is no single inventive concept underlying the plurality of claimed inventions of the present application in the sense of Rule 13.1 PCT. Consequently there is a lack of unity and the groups mentioned above represent independent inventions.

The attention of the applicant is drawn to the fact that claims 1-27 further lack unity for the following reasons :

1. Claims 1-17 refer to methods for preventing tumor cell proliferation or metastasis by treating said cells with heparinase III.

2. Claims 18-19 refer to a method for preparing therapeutic HLGAG fragments for the treatment of a tumor by treating a portion of a tumor with heparinase III.

3. Claims 20-22 refer to methods of treatment of a subject having a tumor by administering a therapeutic HLGAG fragment to said subject.

4. Claims 23-27 refer to compositions comprising a heparinase III and a targeting molecule or a therapeutic HLGAG fragment.

The use of heparinase III (optionally linked to a targeting molecule) for preventing cancer being well known from documents D1 and D4 (see points V-3.1 and V-4 of the present opinion) and HLGAG fragments produced by heparinase III being well-known from documents D2 and D3 and the targeting (see points V-3.1 and V-3.2 of the present opinion), the IPEA fails to see what could be considered as an inventive common concept linking the different groups mentioned above. Therefore, the present application lacks unity and the different groups mentioned above represent independent inventions.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present application refers to methods for preventing proliferation of a tumor or for preventing tumor cell metastasis comprising exposing a tumor cell to an effective amount of (optionally modified) heparinase III, optionally in association with additional anti-cancer drugs. The application also refers to a method for preparing therapeutic agents (HLGAG fragments) for the treatment of a tumor and to compositions comprising heparinase III or therapeutic HLGAG fragments and a targeting molecule for targeting heparinase III to the tumor.

2. **Reference is made to the following documents :**

   D1: WO 96 01648 A (IBEX TECHNOLOGIES R AND D INC ;ZIMMERMANN JOSEPH (US); VLODAVSKY I) 25 January 1996 (1996-01-25)
   D2: WO 94 21689 A (CANCER RES CAMPAIGN TECH ;LYON MALCOLM (GB); GALLAGHER JOHN THOMAS) 29 September 1994 (1994-09-29)
   D3: WO 93 19096 A (CANCER RES CAMPAIGN TECH) 30 September 1993 (1993-09-30)
   D4: WO 95 13830 A (MASSACHUSETTS INST TECHNOLOGY ;CHILDRENS MEDICAL CENTER (US)) 26 May 1995 (1995-05-26)

3. **Lack of novelty; article 33(2) PCT.**

   3.1 The document D1 discloses, inter alia, the fact that selectively removing

heparan sulphate from cell surfaces while leaving the extracellular matrix intact inhibits cell proliferation by down regulating the cell's response to growth factors and that this can be achieved by targeting heparin or heparan sulphate degrading activities to the cell surface. The glycosaminoglycan degrading enzymes comprise heparinase I, II and III. The targeting of said enzymes can be achieved by genetically engineering a ligand binding functionality into heparinase proteins or by physically controlling the localized enzyme concentration through the method of administration. (Abstract). Means of administration are disclosed on p.32 and include topically administration or injection.
Claim 8 discloses a method to diminish the response of a cell to growth factors by directing a glycosaminoglycan degrading enzyme to the surface of targeted cells. Claim 9 specifies that targeting is achieved by incorporating a cell specific ligand binding function and heparin or heparan sulfate degrading activity into a fusion protein having glycosaminoglycan degrading enzyme activity and claim 11 specifies that the ligand is specific to or present in higher concentration in cancer cells as compared with normal cells. Claims 17-28 refer to pharmaceutical compositions comprising a glycosaminoglycan degrading enzyme in combination with a pharmaceutically acceptable carrier and optionally means for targeting the enzyme to cells or tissues (claims 23-25).

Even if the mechanism of action of the heparinase III was not known from the authors of D1, the method disclosed in D1 is the same as the method disclosed in the present application, i.e. targeting a heparinase - which can be heparinase III - to a tumor cell. Since no further indications are given in the methods of the present application, the IPEA assumes that the mere presence of heparinase III at the surface of a tumor cell will be sufficient to prevent proliferation of the tumor cell or metastasis.
Moreover, the IPEA is the opinion that the fusion protein disclosed in D1 can be considered as a modified heparinase III.

Therefore, the subject-matter of claims 1-5, 9, 11-14 and 16-17 can not be considered as novel in the sense of article 33(2) PCT.

The attention of the applicant is drawn to the fact that even if the specific use for the compositions of D1 is not given, some of the compositions claimed in D1 will have the same components as the compositions claimed in the present application. Therefore, claims 23-26 can not be considered as novel in the sense of article 33(2) PCT.

3.2 The document D2 discloses heparan sulphate oligosaccharides having hepatocyte growth factor affinity. The oligosaccharides disclosed in D2 are composed of at least three disaccharide units including one or more internal sequences of an N-sulphated D-glucosamine 6-sulphate residue and an L-iduronic acid residue (Abstract). The oligosaccharide chains disclosed in D1 are obtained by enzymatic partial depolymerisation to the fullest extent of heparan sulphate using heparinase III, followed by size fractioning (p. 5, last paragraph). D2 also claims such an oligosaccharide composition (claims 1-19), methods of isolating low molecular weight oligosaccharides from heparan sulphate proteoglycanes of mammalian cells using heparinase III treatment (claim 28) and the therapeutic use of the oligosaccharide compositions (claims 30-33).

The IPEA is the opinion that even if the compositions of D2 have not been specifically disclosed for use in treatment of cancer, the compositions disclosed in D2 will be the same as the compositions disclosed in claims 23-27 as far as they refer to HLGAG fragments. Therefore, claims 23-27 can not be considered as novel over the teaching of D2 (article 33(2) PCT).

3.3 The document D3 discloses oligosaccharides having growth factor binding affinity. These oligosaccharides can be prepared from glycosaminoglycans such as heparan sulphate and can be used either to activate and stimulate FGF activity or inhibit FGF activity. The use of said oligosaccharides for therapeutic purposes in medicine is also disclosed (Abstract). Some of the oligosaccharides were depolymerised using heparitinase (i.e. heparinase III) (p. 33-34, Depolymerisation of HS to selectively produce sulphated oligosaccharides). Further purifications steps are disclosed (p. 34-38). The use of the oligosaccharides for antitumour treatment is suggested (p. 40, lines 19-21). Claim 11 discloses an oligosaccharide product obtained by

digestion of heparan sulphate by heparitinase, claim 25 discloses a method for obtaining such an oligosaccharide product using heparitinase, claims 33-35 refer to the therapeutical use of such an oligosaccharide product, inter alia for inhibiting cancer cell growth and metastasis and claim 40 refers to a method of treatment, inter alia for inhibiting cancer cell growth and metastasis using an oligosaccharide product according to D3.

Therefore, the subject-matter of claims 23-27 as far as they refer to HLGAG fragments can not be considered as novel over the teaching of D3 (article 33(2) PCT) (see also points 3.1 and 3.2 for the explanations).

4. Lack of inventive step; article 33(3) PCT.

The IPEA is the opinion that the skilled person, knowing from the document D1 that tumor growth or metastasis could be prevented by targeting a heparinase (including heparinase III) to the surface of a tumor cell, would have contemplated to administrate the heparinase orally or to administrate said enzyme in conjunction with other well-known anti-cancer compounds.
Therefore, claims 6, 8 and 15 can not be considered as inventive (article 33(3) PCT).

The IPEA is also the opinion that the treatment of a tumor with heparinase in vitro can also not be considered as inventive. Therefore, claims 7 lacks inventive step in the sense of article 33(3) PCT.

The selection of specific tumor to be treated can also not be considered as inventive as long as this selection is not motivated by a technical purpose. For the moment, the IPEA fails to see such a technical purpose for the selection of the prostate tumor and melanoma. Therefore, claim 10 can not be considered as inventive (article 33(3) PCT).

The attention of the applicant is also drawn to the fact that the document D4 deals with the use of heparinases - including heparinase III - for inhibiting angiogenesis. An application of the methods disclosed in D4 is the treatment of solid tumors. Different ways of administration of heparinase are discussed, including direct

injection in tumors (p. 38-39).
Even if the purpose for administering heparinase III in D4 (inhibiting angiogenesis which is necessary for solid tumor growth and metastasis) is not the same as in the present application, the administration of said heparinase to tumor cells for treating cancer has been suggested in D4. Therefore, the subject-matter of claims 1-17 can not be considered as inventive over the teaching of D4 (article 33(3) PCT).

5. Industrial applicability; article 33(4) PCT.

Claims 4-6, 9, 11, 15 and 20-22 refer to method of treatment of the human or animal body.
The methods of claims 1-3, 8, 10, 12-14 and 16 (partially) can be considered as method of treatment of the human or animal body as long as they are practised in vivo.
For the assessment of the present claims 4-6, 9, 11, 15 and 20-23 (completely) and claims 1-3, 8, 10, 12-14 and 16 (partially) on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

6. Further remarks concerning the application.

6.1 The attention of the applicant is drawn to the fact that, in the light of the prior art, the composition of the HLGAGs at the surface of a cell can greatly vary from one cell type to another. Therefore, the IPEA considers that it is questionable if the HLGAG fragments resulting from heparinase III treatment of every type of tumor cell will result in the generation of HLGAG fragments having a tumor- or metastasis-preventing activity according to the present application.

6.2 Claims 23-27 refer to compositions comprising, inter alia, HLGAG fragments. The IPEA is the opinion that said claims are not clear for the following reasons :

(i) All HLGAG fragments - including those generated by heparinase I or II, for example - will not have an effect for preventing tumor growth or metastasis. The IPEA is the opinion that, faced to all the possible HLGAG fragments covered by claims 23-27, the skilled will not be able to determine those having a tumor growth or metastasis-preventing activity without undue burden of experimentation.

(ii) The attention of the applicant is drawn to the fact that the HLGAG fragments, being products, should be defined in terms of technical features of the product. The IPEA is the opinion that the definition of the HLGAG fragments in term of process steps would also not be considered as sufficiently clear since the method for producing HLGAG fragments in the present application will give very different HLGAG fragments, depending on the nature of the HLGAG at the surface of the cell and the conditions of the heparinase III treatment.

(iii) The compositions of claims 23-27 comprise heparinase III and a targeting molecule. However, in said claims, nothing is said about the link between heparinase III and the targeting molecule. The IPEA is the opinion that the mere presence of a targeting molecule in the compositions of claims 23-27 is not sufficient to target the heparinase III to a specific site.